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(54) Title: **ENDOTHELIAL CELL EXPRESSION PATTERNS**

(57) Abstract: To gain a better understanding of tumor angiogenesis, new techniques for isolating endothelial cells (ECs) and evaluating gene expression patterns were developed. When transcripts from ECs derived from normal and malignant colorectal tissues were compared with transcripts from non-endothelial cells, over 170 genes predominantly expressed in the endothelium were identified. Comparison between normal- and tumor-derived endothelium revealed 79 differentially expressed genes, including 46 that were specifically elevated in tumor-associated endothelium. Experiments with representative genes from this group demonstrated that most were similarly expressed in the endothelium of primary lung, breast, brain, and pancreatic cancers as well as in metastatic lesions of the liver. These results demonstrate that neoplastic and normal endothelium in humans are distinct at the molecular level, and have significant implications for the development of anti-angiogenic therapies in the future.

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CLAIMS

1. An isolated molecule comprising an antibody variable region which specifically binds to an extracellular domain of a TEM protein selected from the group consisting of: 1, 9, 17, 19, and 44, as shown in SEQ ID NO: 196, 212, 230, 232, and 271, respectively.
2. The isolated molecule of claim 1 which is an intact antibody molecule.
3. The isolated molecule of claim 1 which is a single chain variable region (ScFv).
4. The isolated molecule of claim 1 which is a monoclonal antibody.
5. The isolated molecule of claim 1 which is a humanized antibody.
6. The isolated molecule of claim 1 which is a human antibody.
7. The isolated molecule of claim 1 which is bound to a cytotoxic moiety.
8. The isolated molecule of claim 1 which is bound to a therapeutic moiety.
9. The isolated molecule of claim 1 which is bound to a detectable moiety.
10. The isolated molecule of claim 1 which is bound to an anti-tumor agent.

11. A method of inhibiting neoangiogenesis, comprising:
administering to a subject in need thereof an effective amount of an isolated molecule comprising an antibody variable region which specifically binds to an extracellular domain of a TEM protein selected from the group consisting of: 1, 9, 17, 19, 22, and 44, as shown in SEQ ID NO: 196, 212, 230, 232, 238, and 271, respectively, whereby neoangiogenesis is inhibited.
12. The method of claim 11 wherein the subject bears a vascularized tumor.
13. The method of claim 11 wherein the subject has polycystic kidney disease.
14. The method of claim 11 wherein the subject has diabetic retinopathy.
15. The method of claim 11 wherein the subject has rheumatoid arthritis.
16. The method of claim 11 wherein the subject has psoriasis.

17. A method of inhibiting tumor growth, comprising:

administering to a human subject bearing a tumor an effective amount of an isolated molecule comprising an antibody variable region which specifically binds to an extracellular domain of a TEM protein selected from the group consisting of: 1, 9, 17, 19, 22, and 44, as shown in SEQ ID NO: 196, 212, 230, 232, 238, and 271, respectively, whereby growth of the tumor is inhibited.

18. An isolated molecule comprising an antibody variable region which specifically binds to a TEM protein selected from the group consisting of: 9, 17, 19, and 44, as shown in SEQ ID NO: 212, 230, 232, and 271, respectively.

19. The isolated molecule of claim 18 which is a single chain variable region (ScFv).

20. The isolated molecule of claim 18 which is a monoclonal antibody.

21. The isolated molecule of claim 18 which is a humanized antibody.

22. The isolated molecule of claim 18 which is a human antibody.

23. The isolated molecule of claim 18 which is bound to a cytotoxic moiety.

24. The isolated molecule of claim 18 which is bound to a therapeutic moiety.

25. The isolated molecule of claim 18 which is bound to a detectable

moiety.

26. The isolated molecule of claim 18 which is bound to an anti-tumor agent.
27. The isolated molecule of claim 18 which is an intact antibody molecule.
28. An isolated and purified human transmembrane protein selected from the group consisting of: TEM 9, 17, and 19 as shown in SEQ ID NO: 212, 230, and 232, respectively.
29. An isolated and purified nucleic acid molecule comprising a coding sequence for a transmembrane TEM selected from the group consisting of: TEM 9, 17, and 19 as shown in SEQ ID NO: 212, 230, 232, respectively.
30. The isolated and purified nucleic acid molecule of claim 29 which comprises a coding sequence selected from those shown in SEQ ID NO: 211, 229, and 231.
31. A recombinant host cell which comprises a nucleic acid molecule comprising a coding sequence for a transmembrane TEM selected from the group consisting of: TEM 9, 17, and 19 as shown in SEQ ID NO: 212, 230, and 232, respectively.
32. The recombinant host cell of claim 31 which comprises a coding sequence selected from those shown in SEQ ID NO: 211, 229, and 231.
33. A method of inducing an immune response in a mammal, comprising:
administering to the mammal a nucleic acid molecule comprising a coding sequence for a human transmembrane protein selected from the group consisting of: TEM 1, 9, 13, 17, 19, 22, 30, and 44 as shown in SEQ ID NO: 196, 212, 220, 230, 232, 238, 250 and 271, respectively, whereby an immune response to the human transmembrane protein is induced in the mammal.

34. The method of claim 33 wherein the coding sequence is shown in SEQ ID NO: 195, 211, 219, 229, 231, 237, 249, 270.

35. A method of inducing an immune response in a mammal, comprising:

administering to the mammal a purified human transmembrane protein selected from the group consisting of: TEM 1, 9, 13, 17, 19, 22, 30, and 44 as shown in SEQ ID NO: 196, 212, 220, 230, 232, 238, 250 and 271, respectively, whereby an immune response to the human transmembrane protein is induced in the mammal.

36. A method for identification of a ligand involved in endothelial cell regulation, comprising:

contacting a test compound with an isolated and purified human transmembrane protein selected from the group consisting of 1, 9, 13, 17, 19, 30, and 44 as shown in SEQ ID NO: 196, 212, 220, 230, 250, 232 and 271;

contacting the isolated and purified human transmembrane protein with a molecule comprising an antibody variable region which specifically binds to an extracellular domain of a TEM protein selected from the group consisting of: 1, 9, 13, 17, 19, 30, and 44 as shown in SEQ ID NO: 196, 212, 220, 230, 250, 232 and 271, respectively;

determining binding of the molecule comprising an antibody variable region to the human transmembrane protein, wherein a test compound which diminishes the binding of the molecule comprising an antibody variable region to the human transmembrane protein is identified as a ligand involved in endothelial cell regulation.

37. A method for identification of a ligand involved in endothelial cell regulation, comprising:

contacting a test compound with a cell comprising a human transmembrane protein selected from the group consisting of 1, 9, 17, and 19 as shown in SEQ ID NO: 196, 212, 230, and 232;

contacting the cell with a molecule comprising an antibody variable region which specifically binds to an extracellular domain of a TEM protein selected from the group consisting of: 1, 9, 17, and 19 as shown in SEQ ID NO: 196, 212, 230, and 232, respectively;

determining binding of the molecule comprising an antibody variable region to the cell, wherein a test compound which diminishes the binding of the molecule comprising an antibody variable region to the cell is identified as a ligand involved in endothelial cell regulation.

38. A soluble form of a human transmembrane protein selected from the group consisting of: TEM 1, 9, 17, 19, 22, 30 and 44 as shown in SEQ ID NO: 196, 212, 230, 232, 238, 250, and 271, respectively, wherein the soluble forms lack transmembrane domains.

39. The soluble form of claim 38 wherein the soluble form consists of an extracellular domain of the human transmembrane protein.

40. A method of inhibiting neoangiogenesis in a patient, comprising: administering to the patient a soluble form of a human transmembrane protein according to claim 38, whereby neoangiogenesis in the patient is inhibited.

41. A method of inhibiting neoangiogenesis in a patient, comprising: administering to the patient a soluble form of a human transmembrane protein according to claim 39, whereby neoangiogenesis in the patient is inhibited.

42. The method of claim 40 wherein the patient bears a vascularized tumor.

43. The method of claim 41 wherein the patient bears a vascularized tumor.

44. The method of claim 40 wherein the patient has polycystic kidney disease.
45. The method of claim 40 wherein the patient has diabetic retinopathy.
46. The method of claim 40 wherein the patient has rheumatoid arthritis.
47. The method of claim 40 wherein the patient has psoriasis.
48. The method of claim 41 wherein the patient has polycystic kidney disease.
49. The method of claim 41 wherein the patient has diabetic retinopathy.
50. The method of claim 41 wherein the patient has rheumatoid arthritis.
51. The method of claim 41 wherein the patient has psoriasis.
52. A method of identifying regions of neoangiogenesis in a patient, comprising:
administering to a patient a molecule comprising an antibody variable region which specifically binds to an extracellular domain of a TEM protein selected from the group consisting of: 1, 9, 13, 17, 19, 22, 30, and 44, as shown in SEQ ID NO: 196, 212, 220, 230, 232, 238, 250, and 271, respectively, wherein the molecule is bound to a detectable moiety; and
detecting the detectable moiety in the patient, thereby identifying neoangiogenesis.
53. A method of screening for neoangiogenesis in a patient, comprising:

contacting a body fluid collected from the patient with a molecule comprising an antibody variable region which specifically binds to an extracellular domain of a TEM protein selected from the group consisting of: 1, 9, 17, 19, and 44, as shown in SEQ ID NO: 196, 212, 230, 232, and 271, respectively, wherein detection of cross-reactive material in the body fluid with the molecule indicates neoangiogenesis in the patient.

54. A method of screening for neoangiogenesis in a patient, comprising:

contacting a body fluid collected from the patient with a molecule comprising an antibody variable region which specifically binds to a TEM protein selected from the group consisting of: 4, 6, 7, 10, 12, 14, 25, 27, 31, 36, 37, 38, 39, as shown in SEQ ID NO: 202, 206, 208, 214, 218, 223 & 224, 242, 244, 252, 257, 259, 261, and 263, respectively, wherein detection of cross-reactive material in the body fluid with the molecule indicates neoangiogenesis in the patient.

55. A method of promoting neoangiogenesis in a patient, comprising:

administering to a patient in need of neoangiogenesis a TEM protein selected from the group consisting of: 4, 6, 7, 10, 12, 14, 20, 25, 27, 31, 36, 37, 38, 39, and 40, as shown in SEQ ID NO: 202, 206, 208, 214, 218, 223 & 224, 234, 242, 244, 252, 257, 259, 261, 263, and 265, whereby neoangiogenesis in the patient is stimulated.

56. A method of promoting neoangiogenesis in a patient, comprising:

administering to a patient in need of neoangiogenesis a nucleic acid molecule encoding a TEM protein selected from the group consisting of: 4, 6, 7, 10, 12, 14, 20, 25, 27, 31, 36, 37, 38, 39, and 40, as shown in SEQ ID NO: 202, 206, 208, 214, 218, 223 & 224, 234, 242, 244, 252, 257, 259, 261, 263, and 265, whereby the TEM protein is expressed and neoangiogenesis in the patient is stimulated.

57. A method of screening for neoangiogenesis in a patient, comprising:

detecting a TEM protein selected from the group consisting of: 4, 6, 7, 10, 12, 14, 20, 25, 27, 31, 36, 37, 38, 39, and 40, as shown in SEQ ID NO: 202, 206, 208, 214, 218, 223 & 224, 234, 242, 244, 252, 257, 259, 261, 263, and 265, respectively, in a body fluid collected from the patient, wherein detection of the TEM protein indicates neoangiogenesis in the patient.

58. A method of screening for neoangiogenesis in a patient, comprising:

detecting in a body fluid collected from the patient a nucleic acid encoding a TEM protein selected from the group consisting of: 4, 6, 7, 10, 12, 14, 20, 25, 27, 31, 36, 37, 38, 39, and 40, wherein the nucleic acid is selected from the group consisting of those shown in SEQ ID NO: 201, 205, 207, 213, 217, 221 & 222, 233, 241, 243, 251, 256, 258, 260, 262, and 264, respectively, wherein detection of the TEM protein indicates neoangiogenesis in the patient.

59. An isolated and purified nucleic acid molecule which encodes a NEM protein selected from the group consisting of: 14, 22, 23, and 33 as shown in SEQ ID NO: 279, 283, 285, 286, 287, and 289.

60. The nucleic acid molecule of claim 60 wherein the nucleic acid molecule comprises a coding sequence as shown in SEQ ID NO: 278, 282, 284, and 288.

61. A recombinant host cell which comprises a nucleic acid according to claim 60.

62. An isolated and purified NEM protein selected from the group consisting of: 14, 22, 23, and 33 as shown in SEQ ID NO: 279, 283, 285, 286, 287, and 289, respectively.

63. An isolated molecule comprising an antibody variable region which specifically binds to a NEM protein selected from the group

consisting of: 14, 22, 23, and 33, as shown in SEQ ID NO: 279, 283, 285, 286, 287, and 289.

64. A method of inhibiting neoangiogenesis, comprising:

administering to a subject in need thereof an effective amount of a NEM protein selected from the group consisting of: 14, 22, 23, and 33 as shown in SEQ ID NO: 279, 283, 285, 286, 287, and 289, whereby neoangiogenesis is inhibited.

65. A method to identify candidate drugs for treating tumors, comprising:

contacting cells which express one or more TEM genes selected from the group consisting of: 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 19, 20, 21, 22, 24, 25, 27, 28, 29, 30, 31, 33, 35, 36, 37, 38, 39, 41, 42, 44, 45, and 46 as shown in SEQ ID NO: 195, 197, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221 & 222, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 256, 258, 260, 262, 266, 268, 270, 272, and 274, respectively, with a test compound;

determining expression of said one or more TEM genes by hybridization of mRNA of said cells to a nucleic acid probe which is complementary to said mRNA; and

identifying a test compound as a candidate drug for treating tumors if it decreases expression of said one or more TEM genes.

66. The method of claim 66 wherein the cells are endothelial cells.

67. The method of claim 66 wherein the cells are recombinant host cells which are transfected with an expression construct which encodes said one or more TEMs.

68. A method to identify candidate drugs for treating tumors, comprising:

contacting cells which express one or more TEM proteins selected from the group consisting of: 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 19, 20, 21, 22, 24, 25, 27, 28, 29, 30, 31, 33, 35, 36, 37, 38, 39, 41,

42, 44, 45, and 46 as shown in SEQ ID NO: 198, 202, 204, 206, 208, 210, 212, 214, 216, 218, 223 & 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 358, 257, 259, 261, 263, 267, 269, 271, 273, and 275, respectively, with a test compound;

determining amount of said one or more TEM proteins in said cells; and

identifying a test compound as a candidate drug for treating tumors if it decreases the amount of one more TEM proteins in said cells.

69. The method of claim 69 wherein the cells are endothelial cells.

70. The method of claim 69 wherein the cells are recombinant host cells which are transfected with an expression construct which encodes said one or more TEMs.

71. A method to identify candidate drugs for treating tumors, comprising:

contacting cells which express one or more TEM proteins selected from the group consisting of: 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 19, 20, 21, 22, 24, 25, 27, 28, 29, 40, 31, 33, 35, 36, 37, 38, 39, 41, 42, 44, 45, and 46 as shown in SEQ ID NO: 198, 202, 204, 206, 208, 210, 212, 214, 216, 218, 223 & 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 358, 257, 259, 261, 263, 267, 269, 271, 273, and 275 respectively, with a test compound;

determining activity of said one or more TEM proteins in said cells; and

identifying a test compound as a candidate drug for treating tumors if it decreases the activity of of one more TEM proteins in said cells.

72. The method of claim 72 wherein the cells are endothelial cells.

73. The method of claim 72 wherein the cells are recombinant host cells which are transfected with an expression construct which encodes said one or more TEMs.

74. A method to identify candidate drugs for treating patients bearing tumors, comprising:

contacting a test compound with recombinant host cells which are transfected with an expression construct which encodes one or more TEM proteins selected from the group consisting of 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 19, 20, 21, 22, 24, 25, 27, 28, 29, 40, 31, 33, 35, 36, 37, 38, 39, 41, 42, 44, 45, and 46 as shown in SEQ ID NO: 198, 202, 204, 206, 208, 210, 212, 214, 216, 218, 223 & 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 358, 257, 259, 261, 263, 267, 269, 271, 273, and 275, respectively;

determining proliferation of said cells; and

identifying a test compound which inhibits proliferation of said cells as a candidate drug for treating patients bearing tumors.

75. A method to identify candidate drugs for treating tumors, comprising:

contacting cells which express one or more NEM genes selected from the group consisting of: 14, 22, 23, and 33 as shown in SEQ ID NO: 278, 282, 284, and 288, respectively, with a test compound;

determining expression of said one or more NEM genes by hybridization of mRNA of said cells to a nucleic acid probe which is complementary to said mRNA; and

identifying a test compound as a candidate drug for treating tumors if it increases expression of said one or more NEM genes.

76. The method of claim 76 wherein the cells are endothelial cells.

77. The method of claim 76 wherein the cells are recombinant host cells which are transfected with an expression construct which encodes said one or more NEMs.

78. A method to identify candidate drugs for treating tumors,
comprising:

contacting cells which express one or more NEM proteins
selected from the group consisting of: 14, 22, 23, and 33 as shown in SEQ
ID NO: 279, 283, 285, 286, 287, and 289, with a test compound;

determining amount of said one or more NEM proteins in
said cells; and

identifying a test compound as a candidate drug for
treating tumors if it increases the amount of one more NEM proteins in
said cells.

79. The method of claim 79 wherein the cells are endothelial cells.

80. The method of claim 79 wherein the cells are recombinant host
cells which are transfected with an expression construct which
encodes said one or more NEMs.

81. A method to identify candidate drugs for treating tumors, comprising:

contacting cells which express one or more NEM proteins selected from the group consisting of: 14, 22, 23, and 33 as shown in SEQ ID NO: 279, 283, 285, 286, 287, and 289, with a test compound;

determining activity of said one or more NEM proteins in said cells; and

identifying a test compound as a candidate drug for treating tumors if it increases the activity of one more NEM proteins in said cells.

82. The method of claim 82 wherein the cells are endothelial cells.

83. The method of claim 82 wherein the cells are recombinant host cells which are transfected with an expression construct which encodes said one or more NEMs.

84. A method to identify candidate drugs for treating patients bearing tumors, comprising:

contacting a test compound with recombinant host cells which are transfected with an expression construct which encodes one or more NEM proteins selected from the group consisting of 14, 22, 23, and 33 as shown in SEQ ID NO: 279, 283, 285, 286, 287, and 289;

determining proliferation of said cells; and

identifying a test compound which stimulates proliferation of said cells as a candidate drug for treating patients bearing tumors.

85. A method for identification of a ligand involved in endothelial cell regulation, comprising:

contacting a test compound with a human transmembrane TEM protein selected from the group consisting of 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 19, 20, 21, 22, 24, 25, 27, 28, 29, 40, 31, 33, 35, 36, 37, 38, 39, 41, 42, 44, 45, and 46 as shown in SEQ ID NO: 196,

198, 202, 204, 206, 208, 210, 212, 214, 216, 218, 223 & 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 358, 257, 259, 261, 263, 267, 269, 271, 273, and 275;

determining binding of a test compound to the human transmembrane protein, wherein a test compound which binds to the protein is identified as a ligand involved in endothelial cell regulation.

86. A method of inducing an immune response in a mammal, comprising:
administering to the mammal a cell which expresses a transmembrane protein selected from the group consisting of: TEM 1, 9, 13, 17, 19, 22, 30, and 44 as shown in SEQ ID NO: 196, 212, 220, 230, 232, 238, 250 and 271 , respectively, wherein the cell is a recombinant cell which comprises a vector encoding said transmembrane protein, or the cell is a fusion of a dendritic cell and a tumor endothelium cell, whereby an immune response to the human transmembrane protein is induced in the mammal.

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